Impact of Fatty Acid Chain Length and Saturation on Micellarization of Carotenoids during Simulated Digestion of Salad Meal

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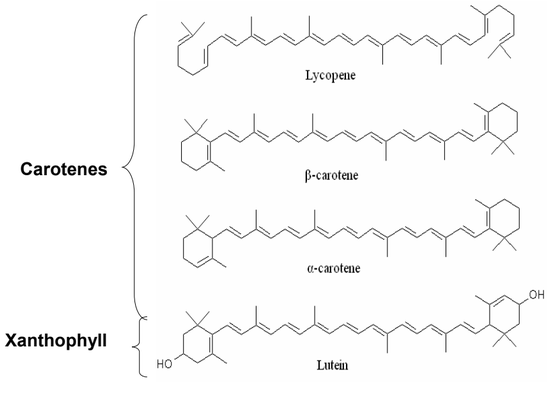
Abstract

The *2005 Dietary Guidelines for Americans* recommend reductions in total fat intake and saturated fat intake to decrease the risk of chronic diseases and obesity. The potential impact of altered fat intake on the bioavailability of pro-vitamin A and non-provitamin A carotenoids has not been systematically investigated. We report the effects of dietary fatty acid composition on carotenoid bioaccessibility using simulated gastric and small intestinal digestion. Triacylglyceride (TG), either a structural lipid or a commercial oil was added to a carotenoid-rich salad meal and the mixture was digested in vitro. Lutein, α-carotene, β-carotene and lycopene in chyme and filtered aqueous fraction (micellar fraction) were measured to determine the efficiency of micellarization of the carotenoids. Micellarization of α-carotene, β-carotene and lycopene was promoted by addition of TG (2.5% wt:wt) to the meal, and dependent on fatty acid chain length of structured lipids (C18:1 > C8:0 > C4:0). In contrast, micellarization of lutein exceeded that of carotenes and lycopene and is independent of presence or type of TG. Micellarization of all four carotenoids during simulated digestion was greater when salad contained coconut oil (rich in saturated fat) instead of canola and safflower oils (rich in unsaturated fats). Initial results also suggest that maximum micellarization of carotenes only required about 1% (wt:wt) test lipid. These data suggest that micellarization of carotenoids is influenced by both chain length and degree of saturation of dietary fatty acids.

Introduction

Carotenoids are lipophilic plant pigments with various biological properties that include pro-vitamin A activity, antioxidant activity, photoprotection of eye and skin, and vitamin A independent regulation of cell signaling and gene transcription. In order to deliver carotenoids and their metabolites to target tissues to modulate such activities, these compounds must be a) released from the food matrix and incorporated into micelles, b) taken up by enterocytes and c) incorporated in chylomicrons and secreted into lymph for distribution to target tissues[1].

The absorption of carotenoids is affected by numerous post-harvesting, physicochemical, dietary, physiological and pathological factors. Dietary lipid is recognized as a potent promoter of carotenoid bioavailability. This is likely associated with the ability of dietary fat to a) provide a "sink" for transfer of carotenoids from food matrix to oil droplets, b) stimulate secretion of bile and pancreatic enzymes, and c) promote the synthesis and secretion of chylomicrons. The effects of quantity and composition of dietary lipids on processes required for the absorption of carotenoids have not been systematically investigated. The goal of this research is to clarify the influences of amount and type of dietary triglycerides (TG) on the following processes : micellarization; uptake of micellarized carotenoids by enterocytes and carotenoid secretion across the basolateral membrane of enterocytes. This study investigated the influence of quantity/composition of dietary TG on micellarization of carotenoids using simulated gastric and small intestinal digestion.



Specific Aims

Aim 1 **Explore the effect of chain length of fatty acids in TG on micellarization of carotenoids by comparing different structural lipids.**

Aim 2 **Examine the impact of degree of saturation of fatty acids in TG on micellarization of carotenoids**

Aim 3 **Investigate the influence of amount of dietary TG on micellarization of carotenoids**

Materials and Methods

1. Test Meal and Lipids

The vegetables and fruits was homogenized to prepare a pureed salad meal and stored in -80˚C. The fatty acid composition was identified by Gas Chromatography.

Table 1: Carotenoid Composition of the test meal

Source	Weight (g)	%	Type of arotenoids*
Tomato	90	35%	lycopene 4-5mg, β-carotene 0.5mg
Carrot	62	25%	β-carotene 5-6mg, α-carotene 2-3mg,
Spinach	50	20%	Lutein 6-7mg, β-carotene 2-4mg
Romaine lettuce	25	10%	lutein 0.5mg, β-carotene 0.5-1mg
Orange pepper	23	10%	lutein 0.5-1mg, β-carotene 0.5-1mg

* The data is from USDA Food Database

Table 2: Fatty acid composition of the test meal

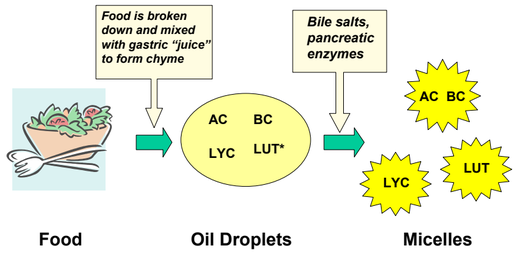
Major fatty acids	Amount (mg/g salad)
18:3n3	0.343
18:2n6	0.433
18:1n7	0.015
18:1n9	0.064
16:0	0.184
Polyunsaturated fatty acids	0.879
Monounsaturated fatty acids	0.083
Saturated fatty acids	0.211
Total Lipids	1.173

Table 3 Test Lipids

Structure lipids	Commercial oils
tri-butyrin (c4:0)	Safflower oil (rich in PUFA)
tri-octanoate (c8:0)	Canola oil (rich in MUFA)
tri-oleate (c18:1)	Coconut oil (rich in SFA)
Conjugated Linoleic Acid (c18:2)	

2. Simulated Digestion

Micellarization of Carotenoids in Gastric and Small Intestinal Digestion



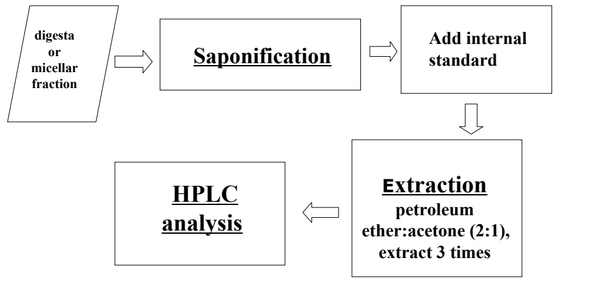
* LUT: Lutein; AC: α-carotene; BC: β-carotene; LYC: Lycopene

The salad meal (3g) with various TG (75μl, except Experiment 4) was digested. The procedure of simulated digestion and isolation of micellar fraction was as described before [2,3].

Digestive stability refers to the degree of recovery of carotenoids in the test material after simulated digestion.The digestive stability was >80% in all experiments.

Efficiency of micellarization refers to the percentage of carotenoid in test material that partition in the filtered aqueous fraction during digestion.

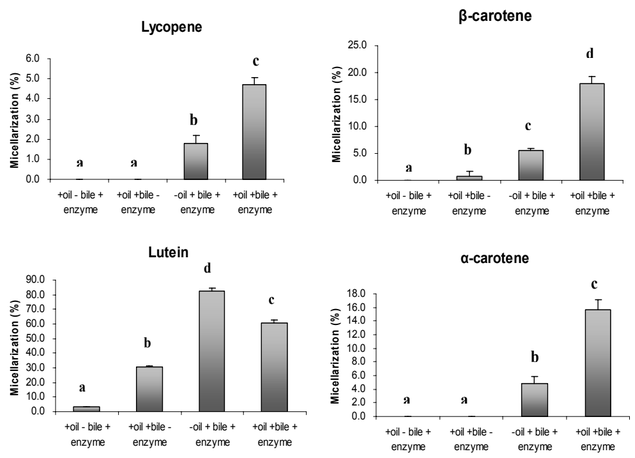
3. Carotenoid extraction and analysis



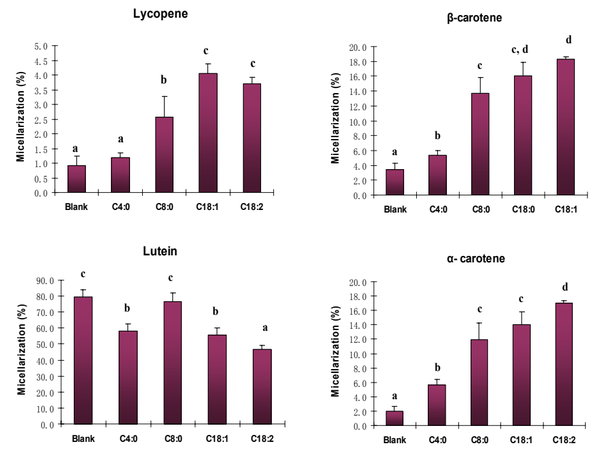
HPLC system: Waters 2695 with Waters 2996 UV-visible PDA detector
Column: Vydac C18 reversed phase column
Solvent system:
solvent A (98% methanol: 2% 1mol/L ammonium acetate)
solvent B (100% ethyl acetate)

Results

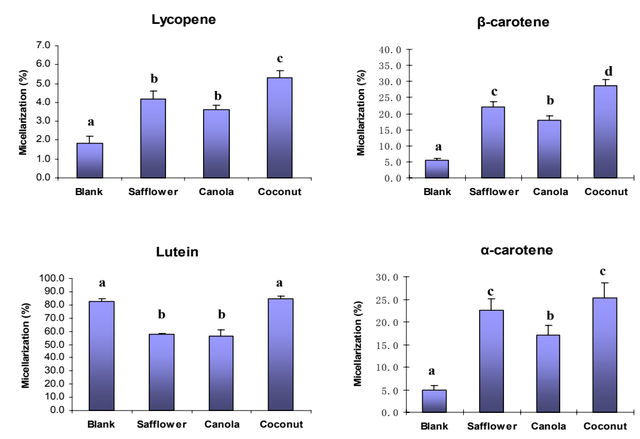
Experiment 1: Bile salts, pancreatic enzymes and dietary TG are required for micellarization of carotenoids from salad meal



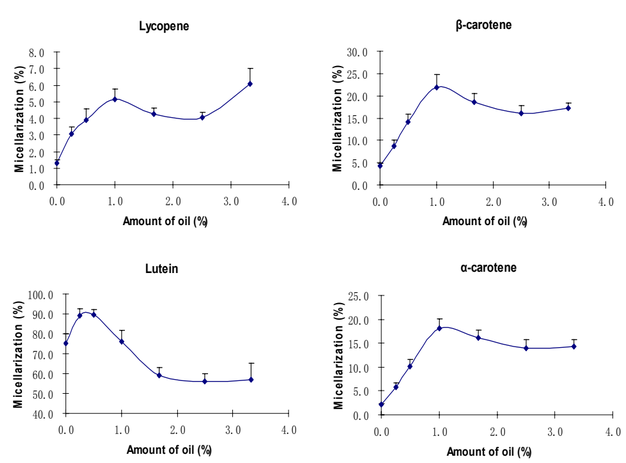
Experiment 2: Micellarization of carotenes, but not lutein, was enhanced with the increase of fatty acid chain length of TG



Experiment 3: Coconut oil enhances the micellarization of carotenes compared to canola oil and safflower oil



Experiment 4: Limited amount of canola oil is required for micellarization of carotenoids



Data were expressed as mean ± sem, One way ANOVA was used to calculate the statistical significance, $p<0.05$

Summary

- **Micellarization of carotenoids during *in intro* digestion of salad meal requires both bile salts and pancreatic digestive enzymes.**
- **Efficiency of micellarization of lutein > α-carotene > β-carotene > lycopene.**
- **TG enhances micellarization of carotenes.**
- **Efficency of micellarization is dependent of chain length and degree of saturation of fatty acids in TG.**
- **Relatively low amount of canola oil (approx. 1%, wt/wt) required for maximum micellarization of carotenoids during digestion of the test meal.**

Conclusion

Use of fat–free dressing with salad decreases micellarization and the bioaccessibility of carotenoids (and possibly other health –promoting fat soluble compounds) during digestion.

References

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[2] Garrett DA, Failla ML, Sarama RJ. Development of an in vitro digestion method to assess carotenoid bioavailability from meals. J Agric Food Chem. 1999; 47(10):4301-4309.

[3] Chitchumroonchokchai C, Schwartz SJ, Failla ML. Assessment of lutein bioavailability from meals and a supplement using simulated digestion and caco-2 human intestinal cells. J Nutr. 2004; 134(9):2280-2286.